JP 2001264284

* NOTICES *

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention has the feature whose biosensor performance improves, when an interaction exists between the alkanethiol molecules which are the modulators of the electrode which fixes the thing about the constructing method of a biosensor, especially an enzyme.
[0002]
[Description of the Prior Art]When an enzyme was fixed in the conventional biosensor, in early stages, the enzyme was included in the matrix of gels, such as polyacrylamide, and on the electrode surface, gel was stiffened and it fixed (for example, 214 Nature(s), 986-988 pages, 1971). However, it was easy to reveal the included enzyme out of the gel matrix, and this method of immobilization was insufficient.
[0003]Next, the enzyme was mixed with cow serum albumin, crosslinking polymerization of both was carried out with glutaraldehyde, and the method of fixing on an electrode surface was devised (for example, Appl.Biochem.Biotechol. nine 95-104-page 1984). However, the enzyme fully needed to examine optimum crosslinking polymerization conditions, an enzyme may be deactivated, and this method was insufficient for applying simple.
[0004]The method which includes an enzyme in conjugate polymer as mentioned above, spots or immerses, and does not fix it in an electrode surface, but is directly fixed in an electrode surface is also devised.
[0005]A carboxyl group is made to form in an electrode surface by carrying out plasma oxidation processing of the graphite electrode, An enzyme is directly fixed in an electrode surface with a water-soluble carbodiimide or glutaraldehyde, and building a biosensor is reported (for example, J.Am.Chem.Soc. 102 4231-4235-page 1980).
[0006]However, by this method, the electrode which fixes an enzyme is restricted to carbon

system electrodes, such as graphite, and it is hard to apply it to a noble metal electrode with a sufficient electrode response.

[0007]Since an enzyme is directly fixed in a noble metal electrode in order to solve this problem, . In the electrode, beforehand, silanizing or after carrying out allylamine processing, the method of carrying out the crosslinking bond of the enzyme with glutaraldehyde is devised. (For example, Anal.Chem.52 volume, 1198-1205 pages, 1980 or 23 Biochemistr(ies), 2203-2210 page 1984).

[0008]However, in order these methods have complicated operation and to use glutaraldehyde as a cross linking agent, By carrying out a crosslinking bond between Silang which embellished the electrode, or between allylamines, inactivation of an enzyme is imitated because the part which an enzyme cannot fix in an electrode produces or the crosslinking bond between the enzymes within an enzyme arises, and there is ********.

[0009]

[Problem(s) to be Solved by the Invention]Thus, since an electrode was fixed, various methods were devised, but it was difficult for there to be merits and demerits and to fix an enzyme for an electrode ornamentation thing and an enzyme regularly as the purpose.

[0010]

[Means for Solving the Problem]In order to solve said technical problem, a biosensor of this invention, A phenomenon which the found-out precious metals and a thiol molecule combine spontaneously is used in recent years. By embellishing a noble metal electrode and making a carboxyl group add to a thiol molecule further in (J.Am.Chem.Soc.105 volume and 4481 page 1983 [for example,]), The crosslinking bond of this and the enzyme is carried out, and a biosensor which can fix an enzyme easily in an electrode surface as the purpose is built.

[0011]According to this invention, in order to embellish a noble metal electrode with a thiol carboxylic acid molecule and to carry out the crosslinking bond of the enzyme to a carboxyl group of this molecule further Crosslinking reaction between modulators, Prevent an intramolecular-branching reaction between molecules of an enzyme, and an intermolecular interaction arises in a thiol molecule which embellished an electrode with what (what is called an alkane thiol) an alkyl group is further introduced into thiol carboxylic acid for, A thiol molecule comes to arrange regularly in an electrode surface, and makes possible regular coordination of an enzyme molecule in an electrode surface from this, and a biosensor with a good response characteristic can be provided.

[0012]

[Embodiment of the Invention]A biosensor according to claim 1 and a manufacturing method for the same of this invention, The alkanethiol molecule which embellishes an electrode surface by inducing intermolecular interactions, such as van der Waals power, on an electrode surface. It is a biosensor, wherein it holds the stacking tendency on the electrode surface of an

alkane thiol and this raises the response performance of a biosensor, Inducing the arrangement regulation nature of the enzyme which inducing an interaction induces the stacking tendency of a modulator and it fixes further between the molecules which embellish an electrode directly realizes high sensitivity-ization of a biosensor.

[0013]A biosensor according to claim 2 and a manufacturing method for the same of this invention, The enzyme which carries out a crosslinking bond to the alkane thiol which embellishes an electrode has oxidation reduction ability specific about an analyte substance, By detecting the current which is the biosensor according to claim 1 detecting the oxidation reduction output of an enzyme by an electrode, catches on an electrode the oxidation reduction output produced with the enzyme, and is produced by electric oxidation reduction. The biosensor which makes an analyte substance detectable is realized.

[0014]A biosensor according to claim 3 and a manufacturing method for the same of this invention, By having functional groups, such as a carboxyl group, in an alkanethiol molecule, and carrying out the crosslinking bond of this functional group and enzyme by a cross linking agent. In order to build a biosensor with the gestalt which laminates a modulator and a substance to be fixed one by one to the electrode which are claim 1 fixing an enzyme in an electrode surface, and the biosensor according to claim 2, and is solid phase, Intramolecular-branching combination between the molecules of a modulator and a substance to be fixed is prevented, and the biosensor which fixed the enzyme as the purpose is realized.

[0015]A biosensor according to claim 4 and a manufacturing method for the same of this invention, They are claim 1 using the electrode which used the precious metals, such as gold and platinum, as the electrode, and embellished the electrode surface by the alkane thiol, claim 2, and the biosensor according to claim 3, A biosensor with a good response is realized by the thing which embellish a conductive good noble metal electrode simple and for which thing use is carried out and an enzyme is fixed in this molecule.

[0016](Embodiment 1) Below, the embodiment of the invention indicated to claim 1, claim 2, claim 3, and claim 4 of this invention is described using drawing 1 and drawing 2.

[0017]Drawing 1 is a figure showing typically the procedure which fixes an enzyme in an electrode.

[0018]This gold electrode is first immersed for 30 minutes using gold which is the precious metals as an electrode into the mercapto alkyl carboxylic acid, 25% ethanol, and 50mM potassium phosphate buffer solution (pH 7.0) of 5 millimols (it abbreviates to mM hereafter) (A-B). Next, an electrode is immersed for 30 minutes into a 10mM chloride 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 50mM potassium phosphate buffer solution (pH 7.0) (B-C). Next, an electrode is immersed for 30 minutes into 10mMN-hydroxysulfo FOKOHAKU acid imide sodium and 50mM potassium phosphate buffer solution (pH 7.0) (C-D). An electrode is immersed into the enzyme solution finally beforehand dialyzed with 50mM

potassium phosphate buffer solution (pH 7.0) (D-E).

[0019]An enzyme is fixable in a noble metal electrode by the above method.

[0020]Since this method immerses the electrode which is solid phase one by one into the alkane thiol, the enzyme, and the cross linking agent solution, it can carry out washing removal of unreacted and the surplus thing in each immersion stage before the next immersion, and can prevent an unnecessary reaction beforehand.

[0021]Drawing 2 is a figure showing the response performance of the biosensor built by the above-mentioned method.

[0022]11-mercaptoundecylic acid and 3-mercaptopropionic acid were used as an electrode as a gold electrode (1.6 millimeters in diameter, product made by BAS), and mercapto alkyl carboxylic acid, and glucose oxidase was used as an enzyme to fix. Glucose oxidase dialyzed and used the solution of 100 micromole.

[0023]The built biosensor trickled glucose into the reaction cell, and measured the current value which impresses the voltage of 0.8 volt in 1 minute, and produces glucose and the hydrogen peroxide generated by the reaction of glucose oxidase by disassembly of hydrogen peroxide.

[0024]The direction of the sensor which embellished the electrode also with the electrode which fixed the same enzyme with 11-mercaptoundecylic acid shows improvement in sensitivity about 3 times as shown in drawing 2.

[0025]The carbon number of the alkyl group between the thiol group of an alkanethiol molecule and a carboxyl group improves intentionally from 5, and the improvement in sensitivity has it to the carbon number 15. [significant]

[0026]An interaction is produced between alkanethiol molecules with the van der Waals power etc. which are induced when an alkyl chain becomes long, and the improvement tendency of the sensor response by this alkyl chain length becoming long brings about the tropism of your kind consideration in the electrode surface of an alkanethiol molecule. Since an enzyme is further fixed on the molecule of this tropism of your kind consideration, it is thought that it fixes while holding arrangement also with a more regular enzyme.

[0027]Since 11-mercaptoundecylic acid of 10 can reveal a high stacking tendency in an electrode surface, also in the glucose oxidase fixed in this, a carbon number holds high regularity, as shown in drawing 2. For this reason, the hydrogen peroxide produced by the enzyme reaction of glucose and glucose oxidase which are analyte substances is efficiently transmitted to an electrode, and it becomes possible to realize high response performance.

[0028]Although the biosensor which fixed glucose oxidase as an example of this invention in drawing 2 was shown, It can apply also to pyranose oxidase, cholesterol oxidase, alcohol oxidase, lactic acid oxidase, glutamate oxidase, ZARUKOSHIN oxidase, the pyruvate oxidase, and xanthine oxidase, without limiting to this.

[0029]Although gold was shown as an example of noble metal electrode material in this invention, it can apply also in a simple substance or alloys, such as platinum, copper, silver, palladium, nickel, iron, titanium, cadmium, indium, and gallium, without limiting to this.
[0030]
[Effect of the Invention]By as mentioned above, the thing for which an alkane thiol with long alkyl chain length is used as a fixed substance of the enzyme which ties a modulator, and also the electrode and enzyme of an electrode according to a biosensor of this invention, and a manufacturing method for the same. Convectivity to the electrode of an enzymatic reaction product is made efficient, and it enables this to provide a biosensor with high response performance.

[Translation done.]